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Design, synthesis, and biological evaluation of novel pan agonists of FFA1, PPARγ and PPARδ

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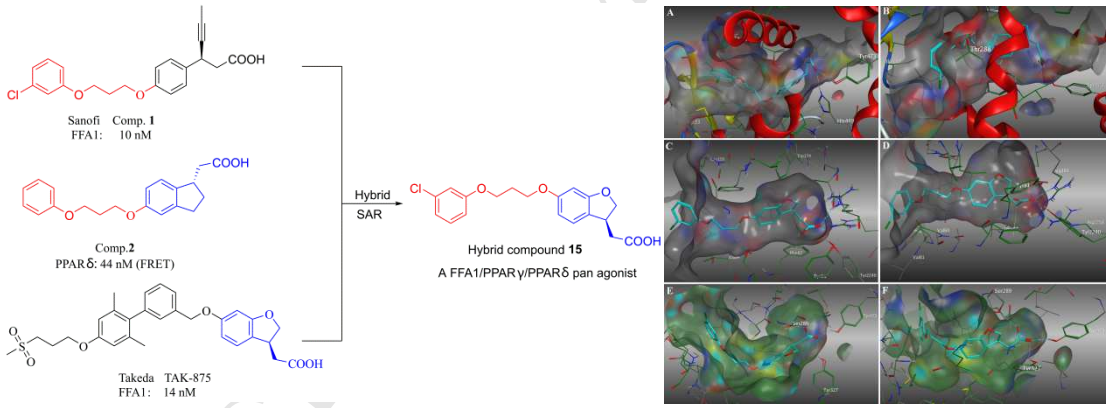
Graphical Abstract



Aiming to develop potent dual PPARs/FFA1 agonists, we have hybrid the Sanofi's FFA1 agonist **1** with PPARδ agonist **2** based on their structural similarity, exemplified by the orally bioavailable FFA1/PPARγ/PPARδ pan agonist **15**.

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Abstract

The free fatty acid receptor 1 (FFA1) and peroxisome proliferator-activated receptors (PPARs) have attracted interest as potent targets for the treatment of metabolic syndrome such as type 2 diabetes. Based on the hypothesis that the dual agonists of PPARs and FFA1 would act as insulin sensitizers and secretagogues by simultaneous activation of PPARs and FFA1, we developed the design strategy to obtain dual PPARs/FFA1 agonist by hybrid FFA1 agonist **1** with PPARδ agonist **2** in consideration of their structural similarity. As expected, systematic exploration of structure-activity relationship and molecular modeling, results in the discovery of lead compound **15**, a pan agonist with relative balanced activities between FFA1, PPARγ and PPARδ. The dose-response relationship studies suggested that the pan agonist **15** suppressed the excursion of blood glucose levels in a dose-dependent manner. During a 5-days treatment in *ob*/*ob* mice, the pan agonist **15** (100 mg/kg) revealed sustained hypoglycemic effect, even proximity to the most advanced FFA1 agonist (TAK-875, 40 mg/kg), which might be attributed to its pan PPARs/FFA1 activities to simultaneous regulate the mechanism of insulin secretion and resistance. These positive results suggest that the dual PPARs/FFA1 agonists such as lead compound **15** might be

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novel therapeutic strategy to modulate the complex pathological mechanisms of type 2 diabetes.

Keywords: FFA1; PPAR; Type 2 diabetes; Hybrid; Pan agonist.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic syndrome characterized by chronic hyperglycemia attribute to insufficient insulin secretion and the peripheral insulin resistance.[1, 2] Agents that improve insulin secretion and resistance such as sulfonylureas and thiazolidinediones are commonly used for the administration of T2DM. However, most of current anti-diabetic drugs are associated with the high risk of hypoglycemia, weight gain, and gastrointestinal side effects.[3-6] Hence, a novel drug that has excellent safety and efficacy in controlling hyperglycemic state would be advantageous.[7-9]

The long-chain free fatty acid receptor 1 (FFA1/GPR40) is mainly expressed in pancreatic β-cells, which promotes the glucose-stimulated insulin secretion without the risk of hypoglycemia.[10-13] Recently, many FFA1 agonists have been reported in literature (**Figure 1**), and several candidates such as TAK-875, AMG-837 and LY2881835 have reached to clinical trials. Readers interested in more structure-activity relationship of FFA1 agonists may refer to our recent reviews.[14, 15] To explore structurally diverse FFA1 agonists, various structure moieties were also introduced in our previous researches.[16-24]

The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that play a critical role in regulating energy metabolism by the modulation of related gene expression.[25] PPARs have three subtypes: PPARα, PPARγ, and PPARδ. The agonists of PPARα or PPARγ revealed potent therapeutic benefit for the T2DM and dyslipidemia.[26, 27] Particularly, PPARγ has been identified as a key regulator for the improvement of insulin resistance, and its agonist rosiglitazone has demonstrated clinical success for the treatment of T2DM.[28] The role of PPARδ is involved in lipid metabolism and inflammation, and thereby plays an important role in obesity, insulin resistance and atherosclerosis.[29-31] Therefore, the dual agonists of PPARs and FFA1 would act as insulin sensitizers and secretagogues by simultaneous activation of PPARs and FFA1. It is worth mentioning that their pharmacophoric features are very similar and their endogenous ligands are free fatty acids.[32] Based on these features, several groups have reported some lead

compound with dual agonistic activities for FFA1 and PPARγ, though their activities are limited to one digit μM.[33-35]

After systematic investigation, we found several interesting compounds in the field of PPARs and FFA1. As shown in **Figure 1**, the Sanofi's FFA1 agonist **1** [14, 15] shared the same scaffold (red lable in **Figure 1**) with the PPARδ agonist **2**.[36] Hence, we speculate that the hybrid of FFA1 agonist **1** and PPARδ agonist **2** would be a rational strategy to obtain the dual PPARs/FFA1 agonist. In consideration of the similarity of acid head between compound **2** and TAK-875, the acid head of TAK-875 was also introduced to the hybrid compounds to maintain the PPARδ activity of compound **2** as much as possible (**Figure 2**). After systematic exploration of structure-activity relationship (SAR) in this hybrid series, the compound **15** was identified as a FFA1/PPARγ/PPARδ pan agonist with potent glucose-lowering effect in *ob*/*ob* mice.

2. Results and Discussion

2.1. Chemistry

The synthetic routes of target compounds **3-15** are shown in **Scheme 1**. The commercially starting material substituted phenols were treated with 1,2-dibromoethane or 1,3-Dibromopropane to afford the intermediates **2a-L**, which were further converted to target compounds **3-14** by Williamson ether synthesis with **3a** [37] and basic hydrolysis. The racemate **4a** was synthesized *via* reported procedures,[37] which was further converted to optically pure **6a** by a novel and simple chiral resolution with R-phenethylamine. Condensation of intermediate **6a** with **2g** by Williamson ether synthesis, followed by basic hydrolysis, furnished the target compound **15**. The compounds **16-18** were synthesized starting from intermediates **2g** and **2L** as summarized in **Scheme 2**. The intermediates **7a-b** were synthesized *via* our previous published procedures.[38] The intermediate **2g** or **2L** was treated with **7a-b** in acetonitrile at the presence of potassium carbonate to give ester in high yield, which was further converted to the desired carboxylic acids **16-18** under hydrolysis conditions of lithium hydrate.

The target compounds **19** and **20** were depicted in **Scheme 3**. The intermediate **9a** was prepared by Knoevenagel condensation of *p*-hydroxy benzaldehyde and Meldrum's acid, followed by treating with sodium borohydride at room temperature. The intermediate **9a** were further derived into deuterated compound **10a** by decarboxylation and esterification. The commercially

starting material **11a** was treated with methyl chloroacetate to obtain **12a**, followed by oxidation with Oxone to provide intermediate **13a**, which was treated with boron tribromide to give **14a**. Subsequently, condensing phenols **10a** or **14a** with **2g** in the presence of potassium carbonate, followed by basic hydrolysis, furnished the target compounds **19** and **20**.

2.2. PPARs/FFA1 agonistic activity and SAR study

In order to explore the SAR of hybrid compounds, the FFA1 agonistic activities were evaluated by a fluorometric imaging plate reader (FLIPR) assay, and the agonistic activities of PPARs were investigated in Gal4 receptor cell-based assay with positive controls GW7647, Rosiglitazone, and GW0742, respectively (**Table 1**). The hybrid compound **4**, a direct analog of selective PPARδ agonist **2**, revealed an approximately 20-fold decrease in PPARδ agonistic activity while the potency on PPARγ has improved at certain degree. Shortening the linker of compound **4** by a methylene provided compound **3**, which led to a significant drop of potency both in PPARγ and PPARδ, suggesting that the length of linker is crucial to the agonistic activity of PPARs. Interestingly, the compound **3** and **4** were almost inactive for FFA1 though their analog Sanofi's FFA1 agonist **1** has highly agonistic activity at nanomolar level (EC_{50} = 10 nM). Methyl scanning in the terminal benzene ring (compounds **5**, **6**, and **7**) indicated that substitution is preferred in the 3-position and tolerated in 4-position for FFA1 agonistic activity, while have little effect on the PPARs. The 2-position substituted derivatives (**5** and **8**) revealed better agonistic activities than parent compound **4** for PPARγ and PPARδ, but there was no significant improvement on the potency of FFA1.

Based on the obtained SAR above, we therefore selected the 3-position as structural modification site in further SAR to maintain a better balance between FFA1, PPARγ and PPARδ (**Table 1**). For the FFA1 and PPARγ, various substituents (compounds **9-13**) were well tolerated in the 3-position. For the PPARδ, the agonistic activity of **9** (3-Cl, 1.75 Å) ＞ **6** (3-Me, 1.80 Å) ＞ **11** (3-*t*Bu) ＞ **13** (3-Ph) suggested that the steric effect in the 3-position might affect the potency of PPARδ, which could be attributed to the limited ligand-binding pocket in this position. Notably, 4-position phenyl substituted derivative **14** revealed improved agonistic activity on PPARα compared to other analogs.

In consideration of the better balance between FFA1, PPARγ and PPARδ, the compound **9**

was selected to provide the optimal enantiomer by chiral separation (**Table 2**). As expected, the optically pure compound **15** (FFA1: 2.38 μM; PPARγ: 1.43 μM; PPARδ: 1.75 μM) revealed a significant improvement on potency compared with the racemate compound **9** (FFA1: 3.07 μM; PPARγ: 1.69 μM; PPARδ: 2.16 μM). With compound **15** as a pan agonist, we decided to focus on the exploration of acid moiety to further extend the SAR (compounds **16**-**20**). The 2-methyl phenoxyacetic acid analog **16** turned out to be more potent than the dihydrobenzofuran series in terms of PPARγ and PPARδ, but the potency on FFA1 was found to be rather low (> 10 μM). Similar to compound **14**, the incorporation of 4-position phenyl (**17**) gave a further increase in the activity of PPARα but unfortunately also significantly decreased potency on PPARγ. The 2-fluoro analog **18** resulted in an approximately 2-fold decrease in PPAR agonistic activity compared to the analog **16**. Consistent with our previously reported FFA1 agonists with 2-fluoro phenoxyacetic acid moiety,[38] compound **18** revealed a significant improvement on the agonistic activity of FFA1. The deuterated phenylpropionic acid (**19**) and phenylsulfonyl acetic acid (**20**), two previleged scaffolds in our reported FFA1 agonists,[22, 23] were also introduced to explore their potential on PPARs and FFA1 by hybrid with compound **15**. Compound **19** indicated an increased FFA1 activity back to the level of compound **9** but, nevertheless, led to 2-fold reduced potency on PPARδ. Interestingly, phenylsulfonyl acetic acid analog **20** appeared to diminish the potency both in PPARs and FFA1, implying that our previously explored SAR could not transfer to this series. After systematic structural optimization, a clear SAR picture of this series was developed and summarized in **Figure 3**.

2.3. Docking study

Based on the crystal complexes of FFA1 (PDB code: 4PHU), PPARγ (2Q8S) and PPARδ (1GWX), we performed an induced-fit docking study of compound **15** to better clarify the SAR of this series. As shown in **Figure 4**, compound **15** fitted very well into the binding pocket of FFA1, PPARδ and PPARγ. In the binding pocket of PPARδ (**Figure 4A** and **4B**), the acidic moiety of compound **15** formed hydrogen-bonding interaction with residues Tyr473 and His449. Moreover, there is an open cavity around the terminal benzene ring, which reasonably explained that the methyl was tolerated in different position (compounds **5**-**7**). Notably, the chlorine is accommodated well into

the small hydrophobic pocket of PPARδ, which could form steric hindrance with bulkier substituents (compounds **11**-**13**). In the binding pocket of FFA1 (**Figure 4C** and **4D**), the carboxylic acid of compound **15** formed strong hydrogen-bonding network with Arg2258 and Arg183, while the α-methylene of acid generate an σ-π interaction with Phe87. Because the steric effect around the 2-position of terminal benzene ring, the substituent was not tolerated in the 2-position (compound **5**). In contrast, the 3-position substituent was preferred due to the hydrophobic interaction with the hydrophobic surface of binding pocket. In the binding site of PPARγ (**Figure 4E** and **4F**), the acidic moiety of compound **15** formed three hydrogen-bonding interactions with Ser289, Tyr473 and Tyr327. Similar to the binding pocket of PPARδ, there is a large hydrophobic cavity around the terminal benzene ring, rationally explained that this area could be occupied by different hydrophobic substituents.

2.4. Dose-response relationship of 15 in normal mice

Based on the better balance between FFA1, PPARγ and PPARδ, compound **15** (10, 30 and 100 mg/kg) was selected to evaluate its hypoglycemic effect and dose-response relationship. As shown in **Figure 5**, compound **15** (10, 30 and 100 mg/kg) suppressed the excursion of plasma glucose levels in a dose-dependent manner in normal mice, with a decrease in the $AUC_{0-120min}$ values of 1.1%, 8.1% and 18.6%, respectively. Moreover, compound **15** revealed significantly hypoglycemic effect at the dose of 100 mg/kg though it is still inferior to TAK-875 (20 mg/kg, -26.9% $AUC_{0-120min}$).

2.5. Hypoglycemic effects of 15 in ob/ob mice

To further investigate hypoglycemic effect in diabetic state, compound **15** was evaluated for its therapeutic potential to improve glucose tolerance in *ob*/*ob* mice, a type 2 diabetes model that develops obese and insulin resistance.[39, 40] In this research, TAK-875 (40 mg/kg), compound **15** (100 mg/kg), or vehicle was orally administered to *ob*/*ob* mice twice daily for five days, and the oral glucose tolerance tests (OGTTs) were determined on day 0 and 5. As shown in **Figure 6**, the acute administration of TAK-875 and compound **15** resulted in a 13.1% and 7.6% reduction on $AUC_{0-120min}$ of glucose at the day 0 of treatment. On day 5, TAK-875 or compound **15** provided robust glucose-lowering effects, with a reduction in the $AUC_{0-120min}$ values of 20.2% and 17.3%,

respectively. These results indicated that the glucose-lowering effect of compound **15** could be maintained and even improved throughout the treatment without the indication of desensitization or tachyphylaxis. Notably, at the 5th days of treatment, the hypoglycemic effect of compound **15** (100 mg/kg, -17.3% $AUC_{0-120min}$) was similar to that of TAK-875 (40 mg/kg, -20.2% $AUC_{0-120min}$). This positive result might be attributed to the dual effects of compound **15** to improve the insulin secretion and sensitivity by simultaneous activation of FFA1, PPARγ and PPARδ.

3. Conclusion

Aiming to develop potent dual PPARs/FFA1 agonists, we have hybrid the Sanofi's FFA1 agonist **1** with the PPARδ agonist **2** based on their structural similarity, exemplified by the orally bioavailable FFA1/PPARγ/PPARδ pan agonist **15**. The important aspects that led to the identification of lead compound **15** were as follows: (1) the hybrid strategy not only maintained the potency on FFA1 and PPARδ to some extent, but also unexpectedly increased the activity of PPARγ; (2) systematic SAR and molecular modeling studies allowed us to explore a better balance of potency between FFA1, PPARγ and PPARδ. The dose-response relationship studies indicated that compound **15** reduced plasma glucose levels in a dose-dependent manner. During a 5-days treatment in *ob*/*ob* mice, compound **15** (100 mg/kg) revealed sustained glucose-lowering effects, even proximity to that of TAK-875 (40 mg/kg), which might be attributed to its pan PPARs/FFA1 agonistic activities to simultaneous regulate the mechanism of insulin secretion and resistance. All of these results suggested that pan agonist **15** was meaningful for further evaluation as a tool compound, and the information acquired from the SAR and molecular modeling studies might help to explore more competitive FFA1/PPARγ/PPARδ pan agonist.

4. Experimental section

4.1. General chemistry

All starting materials, reagents and solvents were obtained from commercial sources and used without further purification unless otherwise indicated. Purifications by column chromatography were carried out over silica gel (200-300 mesh) and monitored by thin layer chromatography performed on GF/UV 254 plates and were visualized using UV light at 254 and 365 nm. NMR spectra were recorded on a Bruker ACF-300Q instrument (300 MHz for ^{1}H NMR and 75 MHz for

^{13}C NMR spectra), chemical shifts are expressed as values relative to tetramethylsilane as internal standard, and coupling constants (*J* values) were given in hertz (Hz). LC/MS spectra were recorded on a Waters liquid chromatography-mass spectrometer system (ESI). Optical rotation was determined on a JASCO P-1030 polarimeter. Elemental analyses were performed by the Heraeus CHN-O-Rapid analyzer. Compound **2**[36] and TAK-875[37] were synthesized as previously reported.

The physical characteristics, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis data for all intermediates and target molecules, were reported in the supporting information.

4.2. Ca^{2+} influx activity of CHO cells expressing human FFA1 (FLIPR Assay)

CHO cells stably expressing human FFA1 were seeded into 96-well plates at a density of 15K cells/well and incubated 16 h in 5% CO_2 at 37 °C. After, the culture medium in the wells was removed and washed with 100 μL of Hank's Balanced Salt Solution. Then, cells were incubated in loading buffer (containing 2.5 μg/mL fluorescent calcium indicator Fluo 4-AM, 2.5 mmol/L probenecid and 0.1% fatty acid-free BSA) for 1 h at 37 °C. Various concentrations of test compounds or γ-linolenic acid (Sigma) were added into the cells and the intracellular calcium flux signals were monitored by FLIPR Tetra system (Molecular Devices). The agonistic activities of test compounds on human FFA1 were expressed as [(A−B)/(C−B)]×100% (increase of the intracellular Ca^{2+} concentration (A) in the test compounds-treated cells and (B) in vehicle-treated cells, and (C) in 10 μM γ-linolenic acid-treated cells). EC_{50} value of tested compound was obtained with Prism 5 software (GraphPad).

4.3. Cell-based GAL4 transactivation assay for PPARγ and PPARδ

For transfection, HEK293 cells were plated onto 96-well plates at 5×10^4 cells/well in DMEM (Gibco) without antibiotics 1 day prior to transfection. Transfections were performed using FuGENE HD Transfection Reagent (Roche) according to the manufacturer's protocol. The cells were transiently transfected with 25 ng/well pBIND-PPARδ or PPARγ, 25 ng/well pG5Luc, and 0.15 μl/well FuGENE HD. After transfection for 24 hours, various concentrations of test compounds or positive controls were added into the cells and incubated for 18 hours. Cells from each well were lysed with 20 μl lysis buffer, and each well was added 30 μl Luciferase Assay

Reagent II. The firefly and renilla luciferase signal is assayed by Promega's Dual Luciferase Reporter Assay System. Envision is used as the luminometer. The data values are normalize by dividing the firefly signal with renilla signal. "F/R" means "Firefly/Renilla". This normalization eliminates the discrepancy of different cell number and transfection efficiency in each well. The %Activation value is calculated by the following equation: %Activation = [(X-Min)/(Max-Min)]×100% (X is the "F/R" value in each concentration point. Min is the mean "F/R" value from no compound control. Max is the mean "F/R" value from reference compound control). EC_{50} value of tested compound was obtained with Prism 5 software (GraphPad).

4.4. Cell-based GAL4 transactivation assay for PPARα

For transfection, HepG2 cells were plated onto 96-well plates at 6×10^4 cells/well in MEM (Gibco) without antibiotics 1 day prior to transfection. The similar protocol was used as described for the cell-based GAL4 transactivation assay for PPARγ and PPARδ.

4.5. Molecular modeling studies

MOE (version 2014.0901, The Chemical Computing Group, Montreal, Canada) was used to perform the docking modeling based on the reported crystal structure of FFA1 (PDB ID: 4PHU), PPARγ (2Q8S) and PPARδ (1GWX). Prior to molecular docking, other crystallized ligands and water were removed, and the obtained protein was performed by Protonate 3D prior to the Gaussian Contact surface was draw around the binding pocket of ligand. Subsequently, the binding pocket with Gaussian Contact surface was isolated. Compound **15** was induced-fit docked into the binding pocket and ranked with London dG scoring function. For energy minimization in binding pocket, MOE Forcefield Refinement was performed and ranked with London dG scoring function. The binding free energy calculations in the docking studies of positive controls and compound **15** were represented as ΔG value.

4.6. Animals and statistical analysis of the data

Male ICR mice (18-22 g) were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China), and six weeks old male *ob*/*ob* mice were purchased from Model Animal

Research Center of nanjing university (Jiangsu, China). All animals were acclimatized for 1 week before the experiments. The animal room was maintained under a constant 12 h light/black cycle with temperature at 23 ± 2 ℃ and relative humidity 50 ± 10% throughout the experimental period. Mice were allowed ad libitum access to standard pellets and water unless otherwise stated, and the vehicle used for drug administration was 0.5% sodium salt of Carboxy Methyl Cellulose aqueous solution for all animal studies. All animal experimental protocols were approved by the ethical committee at Guangdong Pharmaceutical University and conducted according to the Laboratory Animal Management Regulations in China and adhered to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication NO. 85-23, revised 2011).

Statistical analyses were performed using GraphPad InStat version 5.00 (San Diego, CA, USA). General effects were analyzed by Student's t test or using a one-way ANOVA with Tukey's multiple-comparison post hoc test.

4.7. Oral Glucose Tolerance Test in male ICR mice

Normal male ICR mice after adaptation one week were fasted for 12 h, weighted, bled *via* the tail tip, and randomized into 5 groups (n = 6 per group). Compound **15** (10, 30, and 100 mg/kg), TAK-875 (20 mg/kg), or vehicle (0.5% methylcellulose aqueous solution) was orally administered 30 min before oral glucose load (3 g/kg). Blood samples were collected immediately at -30 min (just before drug administration), 0 min (just before glucose challenge), and 15, 30, 60 and 120 min after glucose load. The blood glucose was measured by plasma glucose test strips (SanNuo ChangSha, China).

4.8. Oral Glucose Tolerance Test in male ob/ob mice

The male *ob*/*ob* mice were dosed twice daily with the vehicle (0.5% methylcellulose aqueous solution), TAK-875 (40 mg/kg) or compound **15** (100 mg/kg) by gavage administration for 5 days. Mice were dosed at fixed time daily, and the OGTTs were performed on days 0 and 5 of treatment. Mice were fasted overnight prior to treatment with a single doses of vehicle, TAK-875 (40 mg/kg) or compound **15** (100 mg/kg) and subsequently dosed orally with glucose aqueous solution (3 g/kg) after 30 min. Mice were bled via tail tip immediately before drug administration (-30 min), before

glucose challenge (0 min), and at 15, 30, 60 and 120 min post-dose and the blood glucose was measured by blood glucose test strips (SanNuo ChangSha, China).

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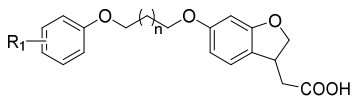
Table 1: *In vitro* activities of the human PPARs and FFA1 [a]

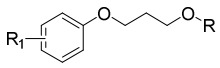


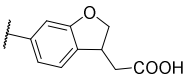
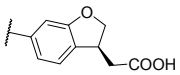
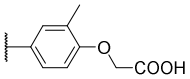
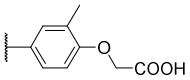
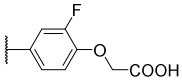
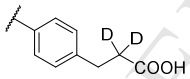
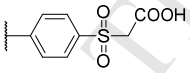
Compd.	R_1	n	*h*FFA1 EC_{50} (μM)	*h*PPARα (LBD)-GAL4 EC_{50} (μM)	*h*PPARγ (LBD)-GAL4 EC_{50} (μM)	*h*PPARδ (LBD)-GAL4 EC_{50} (μM)
TAK-875			0.045	ND	ND	ND
GW7647			ND [b]	0.007	ND	ND
Rosiglitazone			ND	ND	0.12	ND
GW0742			ND	ND	ND	0.013
2			> 10	> 10	> 10	0.17
3	H	0	> 10	> 10	7.75	7.93
4	H	1	> 10	> 10	5.26	3.26
5	2-CH_3	1	> 10	> 10	2.02	1.93
6	3-CH_3	1	3.59	> 10	1.75	2.42
7	4-CH_3	1	7.62	> 10	1.81	1.95
8	2-F	1	> 10	> 10	2.67	2.09
9	3-Cl	1	3.07	> 10	1.69	2.16
10	3-Br	1	3.45	> 10	1.67	2.37
11	3-*t*Bu	1	3.02	> 10	1.75	4.84
12	3-*t*Bu	0	3.85	> 10	2.03	9.57
13	3-Ph	1	2.76	> 10	1.85	5.49
14	4-Ph	1	4.26	7.31	2.97	1.98

[a] EC_{50} value represent the mean of three determinations, which is the concentration giving 50% of the maximal activity determined for the tested compound.

[b] ND: not determined.

Table 2: *In vitro* activities of designed compounds with various acid moieties [a]



Compd.	R_1	R	*h*FFA1 EC_{50} (μM)	*h*PPARα (LBD)-GAL4 EC_{50} (μM)	*h*PPARγ (LBD)-GAL4 EC_{50} (μM)	*h*PPARδ (LBD)-GAL4 EC_{50} (μM)
TAK-875			0.045	ND	ND	ND
GW7647			ND [b]	0.007	ND	ND
Rosiglitazone			ND	ND	0.12	ND
GW0742			ND	ND	ND	0.013
2			> 10	> 10	> 10	0.17
9	3-Cl		3.07	> 10	1.69	2.16
15	3-Cl		2.38	> 10	1.43	1.75
16	3-Cl		> 10	> 10	1.54	1.73
17	4-Ph		> 10	5.26	4.56	1.52
18	3-Cl		8.45	> 10	3.17	3.35
19	3-Cl		3.28	> 10	1.92	4.56
20	3-Cl		> 10	> 10	> 10	> 10

[a] EC_{50} value represent the mean of three determinations, which is the concentration giving 50% of the maximal activity determined for the tested compound.

[b] ND: not determined.

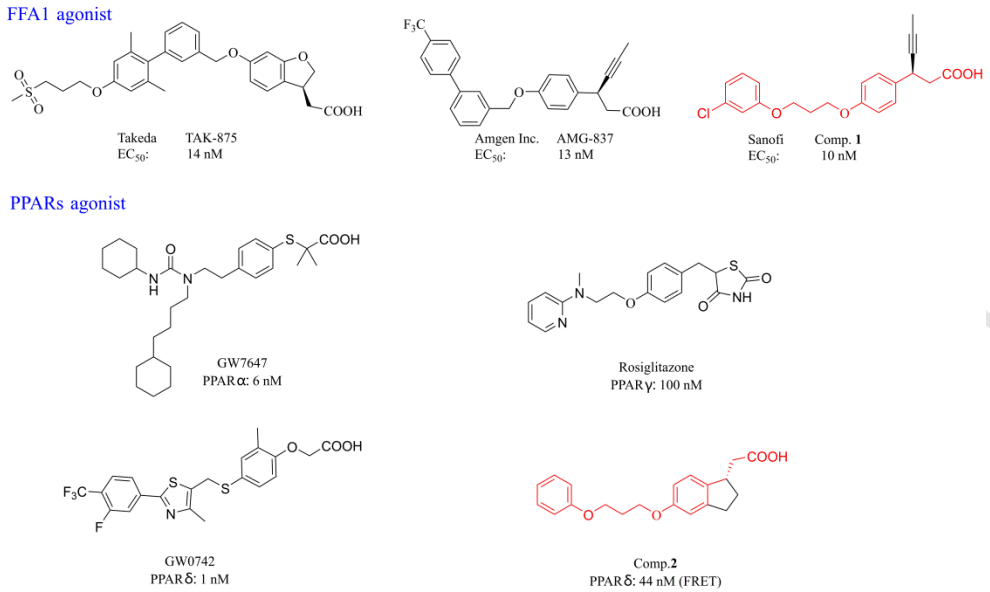


Figure 1: Structure of FFA1 or PPARs agonists, the common scaffold was labeled in red.

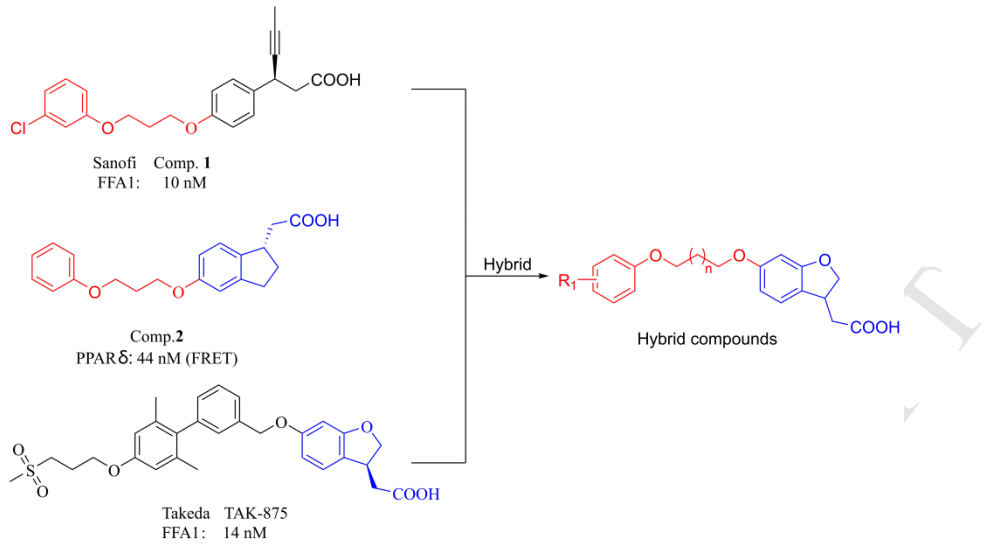
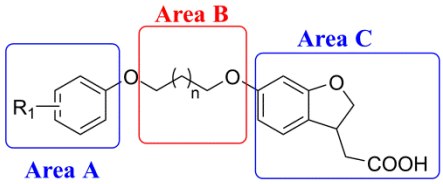


Figure 2: Our strategy to obtain dual PPARs/FFA1 agonists by hybrid the common scaffold of PPARs and FFA1 agonists.



Area A: For FFA1, *ortho*-substituents are not tolerated, and preferred in the *meta*-position. Various substituents were well tolerated in the *meta*-position.

For PPARα, *para*-position phenyl improved the potency while the smaller methyl and hydrogen have little affect on activity.

For PPARγ, small hydrophobic substituent was preferred in terminal benzene ring.

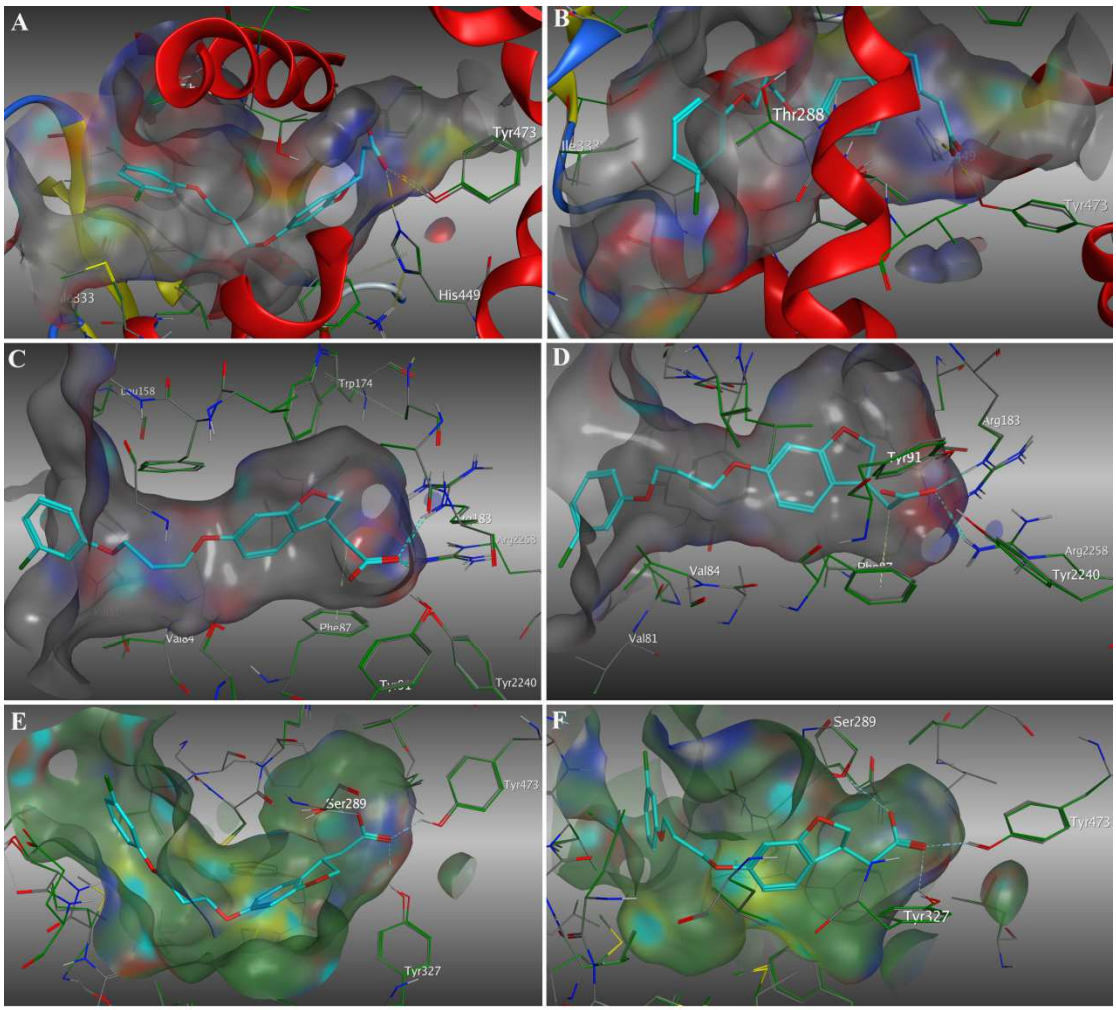
For PPARδ, as the size of *meta*-position substituent increases the potency decreases (Cl > Me > tBu > Ph).

Area B: This area have a strict requirement for the length of linker and conformational flexibility. Five-atom length was preferred for the agonistic activity of FFA1, PPARγ and PPARδ.

Area C: The carboxylate moiety, form hydrogen bonding network with FFA1 and PPARs, was crucial for activity. Once the hydrogen bonding network was disturbed by unfavorable conformation, the agonistic activity will be significantly reduced.

The dihydrobenzofuran acid moiety revealed the best balance between FFA1, PPARγ and PPARδ, especially for its S optimal enantiomers. The phenoxyacetic acid moiety tend to improve the potency of PPARγ and PPARδ, but adversed to the FFA1. The deuterated phenylpropionic acid reduced the activity of PPARδ, and phenylsulfonyl acetic acid appeared to diminish the potency both in PPARs and FFA1.

Figure 3: Structure-activity relationship summary.



G	PPARδ ΔG Value	FFA1 ΔG Value	PPARγ ΔG Value
GW0742	-14.4506		
TAK-875		-16.1973	
Rosiglitazone			-9.8374
Compound **15**	-7.6885	-7.5681	-7.0846

Figure 4: The frontal view and side-view in docking model of compound **15** (cyan carbon) in crystal structure of PPARδ (1GWX, **A** and **B**), FFA1 (4PHU, **C** and **D**) and PPARγ (2Q8S, **E** and **F**). Key residues are labeled in white, and interactions are represented by dashed lines. G represents the binding free energy calculations in the docking studies of positive controls and compound **15**, respectively.

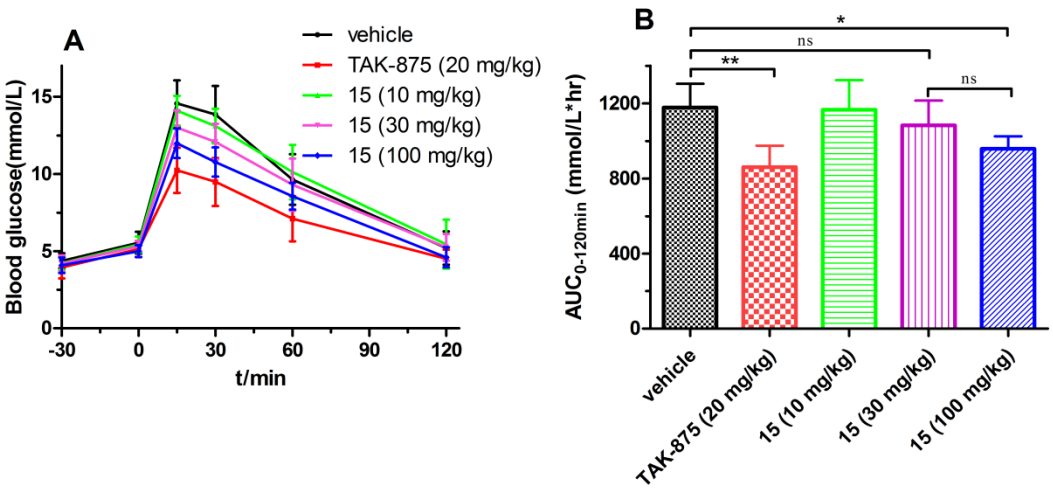


Figure 5: Dose–response relationship of compound **15** explored in normal mice. (A) represented time-dependent changes of blood glucose levels after oral administration of compound **15**, followed by oral glucose load (3 g/kg), respectively. (B) represented $AUC_{0-120min}$ of plasma glucose levels. Results are mean ± SD (n = 6 per group). *p≤ 0.05 and **p≤0.01 was analyzed using a one-way ANOVA with Tukey's multiple-comparison post hoc test.

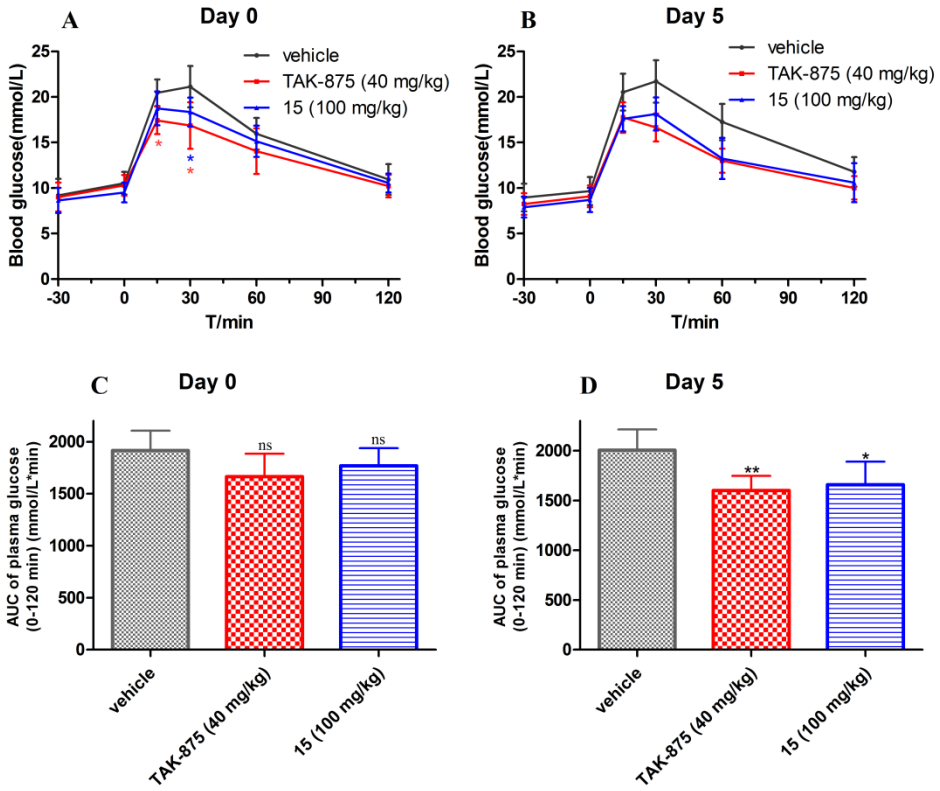
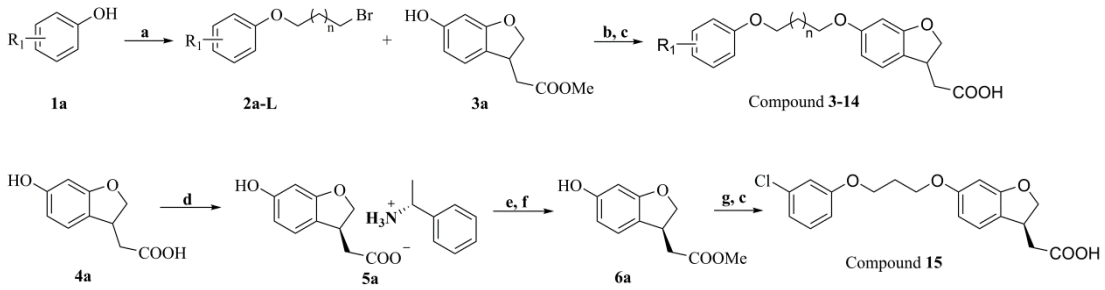
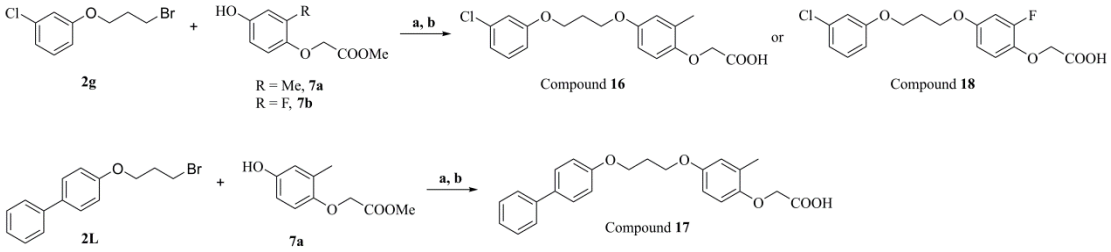


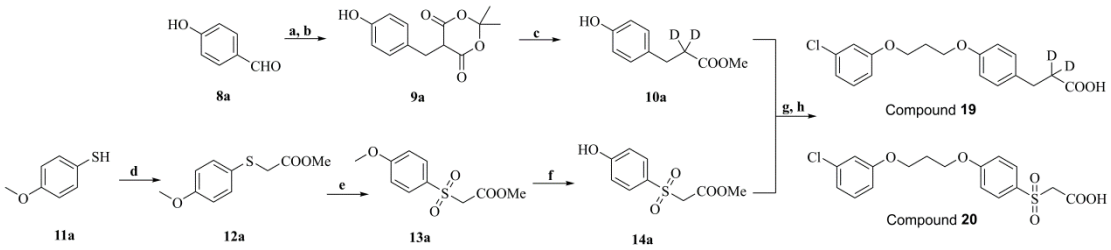
Figure 6: Effects of compound **15** on plasma glucose levels and corresponding $AUC_{0-120min}$ of glucose during an OGTT in fasting *ob*/*ob* mice after five days treatment. TAK-875, compound **15**, or vehicle was orally administered to *ob*/*ob* mice twice daily for 5 days, and the OGTTs were determined on treatment day 0 and 5. Values are mean ± SD (n = 6 per group). *P≤0.05 and **p≤0.01 compared to vehicle mice by Student's t test.



Scheme 1. Synthesis of compounds **3-15**. Reagents and conditions: (a) 1,2-dibromoethane or 1,3-Dibromopropane, K_2CO_3, acetone, 45 °C, 12 h; (b) K_2CO_3, acetonitrile, KI, 45 °C, 12 h; (c) $LiOH \cdot H_2O$, THF/MeOH/H_2O, r.t., 4 h; (d) R-Phenethylamine, acetone, reflux; (e) 1 M HCl; (f) H_2SO_4, MeOH, reflux; (g) **2g**, K_2CO_3, acetonitrile, KI, 45 °C, 12 h.



Scheme 2. Synthesis of compounds **16-18**. Reagents and conditions: (a) K_2CO_3, acetonitrile, KI, 45 °C, 12 h; (b) $LiOH \cdot H_2O$, THF/MeOH/H_2O, r.t., 4 h.



Scheme 3. Synthesis of target compounds **19**-**20**. Reagents and conditions: (a) Meldrum's acid, water, 75 °C, 2 h; (b) $NaBH_4$, r.t.; (c) D_2O, DMF, 100 °C, 12 h, then MeOH, H_2SO_4, reflux, 3 h; (d) Methyl chloroacetate, K_2CO_3, acetone, reflux, 2 h; (e) Oxone, MeOH, H_2O, 0 °C to r.t.; (f) BBr_3, CH_2Cl_2; (g) **2g**, K_2CO_3, acetonitrile, KI, 45 °C, 12 h; (h) $LiOH \cdot H_2O$, THF/MeOH/H_2O, r.t., 4 h.

Highlights

- A therapeutic strategy that simultaneous targets insulin secretion and resistance.
- FFA1/PPARγ/PPARδ pan agonist was identified by the hybrid of FFA1 and PPARδ agonists.
- Compound **15** revealed relative balanced activities between FFA1, PPARγ and PPARδ.
- Compound **15** suppressed the excursion of glucose levels in a dose-dependent manner.
- Compound **15** exhibited similar glucose-lowering effect as TAK-875 after 5-days treatment.